



Controls

OBJECTIVE

To ascertain whether the plaque detection system is working.

BACKGROUND

This test is designed to ensure that plaques come from the intended source.

APPROXIMATE TIME NEEDED

Setting Up Plates: ~**5 minutes**

Allowing the Plates to Solidify: ~**20 minutes**

Incubation: ~**24 hours**

MATERIALS NEEDED

Equipment

- Pipettors, micro- and serological
- Microwave
- Vortex
- 37°C Incubator

Consumables/Reagents (See online media preparation guides)

- *Mycobacterium smegmatis* plating stock (0.5 mL/plate)
- Top agar/MBTA plating mixture (4.5 mL/plate)
- 15 mL conical tubes
- Serological pipettes
- Appropriate micropipette tips
- Agar plates

HELPFUL TIPS

- Always run a negative control.
- Top agar/MBTA plating mixture is prepared by mixing 7H9 (w/ CaCl₂ and other host-specific additives) and stock MBTA in a 1:1 ratio.

PROCEDURES

Positive Control

1. Follow procedures as negative control shown on the next page, but add a 10 µL of a known phage sample to the plating mix.
2. For phage novices, the inclusion of positive controls is **not advocated**. If the purpose for a positive control is to determine whether this system will detect plaques, the current track record of 2400+ phages is a recognizable substitute. Bacterial contamination can be easily recognized and reconciled. Phage contamination can derail a phage isolation or purification project. Remember, there are lots of phage pictures available on www.phagesdb.org.

Negative Control

1. Bring one plate to room temperature. Label as negative control (NC) with initials and draw a 3 cm circle on the plate bottom.
2. Pipet 0.5 mL of *smeg* into a tube.
3. Prepare the top agar:
 - a. Heat the MBTA in a microwave, interrupting and shaking periodically, until it bubbles and no visible solid chunks remain.
 - b. Add an equal volume of room temperature 7H9 w/ + 2 mM CaCl₂ (and any other host-specific additives) to the hot MBTA. Mix until well combined. This will dilute the CaCl₂ to an appropriate final concentration (1 mM) as well as cool the MBTA to a usable temperature. Use the top agar immediately to prevent chunking.
4. While the top agar is still very warm, use a serological pipette to add ~4.5 mL of it to the tube containing *M. smegmatis*.
5. Pour the warm sample onto a **labeled** agar plate. Immediately, but gently, swirl the plate in a circular pattern to spread the agar evenly across the surface of the plate. Allow the plates to **sit still** after swirling until the agar has solidified (generally ~15 minutes).
6. Drop 10 µL of phage buffer on top agar surface in the circle drawn in step #1.
7. Invert once buffer has been absorbed (in about 20 – 25 minutes).
8. Incubate at 37°C overnight.
9. Observations:
 - a. Smooth, clean bacterial lawn: negative control is good.
 - b. If the bacterial lawn is smooth and clean, but the circle has lysis or plaques, the buffer used is contaminated.
 - c. If plaques in lawn outside the circle are identified, either the *smeg* or the top agar is contaminated with phage. Obtain a new culture.
10. Discard any contaminated materials and repeat procedures.